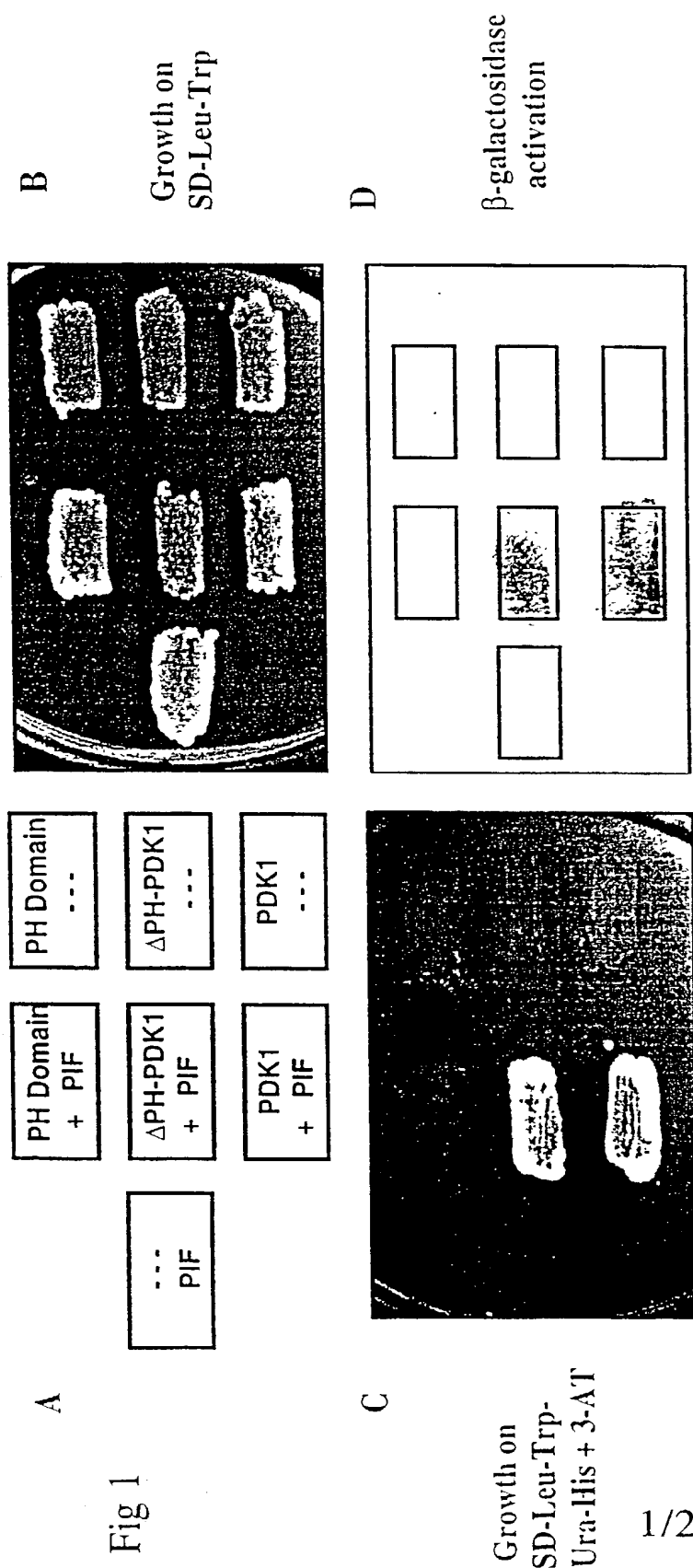


Fig 1



		Region A		Region B			
908	PRK2	EDVKKHPPFRLLIDWSALMDKKVKPPFTPTIRGREDVSNFDDFTSEAPILTPP	---	REPRILSEEEQEM	---	FRDFDYIADMC	984
403	PRK1	EDVKKQPPFRITGWEALFAERTPPPFPPTSGRTDVSNFDEFTGEAPTLPPR	---	DARPLFAAEQAA	---	FLDFDYLACGC	479
400	PKBα	KETIMQHRFFAGLVQHYVYKKLSPPFKPQVTSSETDTRYFDLEFTQMITITPPDDQD	---	SMECVLSERRP	---	HFQQFSYSASTA	480
321	p70S6k	GEVQAHPPFRHIMWELTAKKVEPPFKKLQSEEDVSLFDSKFTRTPTMDSE	---	DDSTLSESAQV	---	FLGFTYLA PSV	395
347	SGK	MEIKSHVFFSLIMDDLAKKKITPPFNPNVSGPNRHRHFDPEFTEEPVPNSIGKSP	---	SVLELVIA SVKEAAEA	---	FLGFSYAPPTD	428
510	PKCζ	SDIKSHAFFRSIDMLLEKKQALPPFQEQITDDYGDNFDTTETSEPVQLTPPD	---	PEDALKRIQSE	---	PEGFYINPLL	585
589	PKCα	RDVREHAFFRIDWEKLENRELPPEFKPKVCG-KGAENFDKFTRGQPLTPPP	---	POLYANINIQSD	---	PEGFSYINPQF	663
290	PKBβ	SDAKTHKGFATTDMIAVQKVEAPPFKFGRSGDTSNFDDYEEELIRV	---	SNIDCAKEK	---	FGEF	351

# A glutathione-Sepharose "Pull Down"

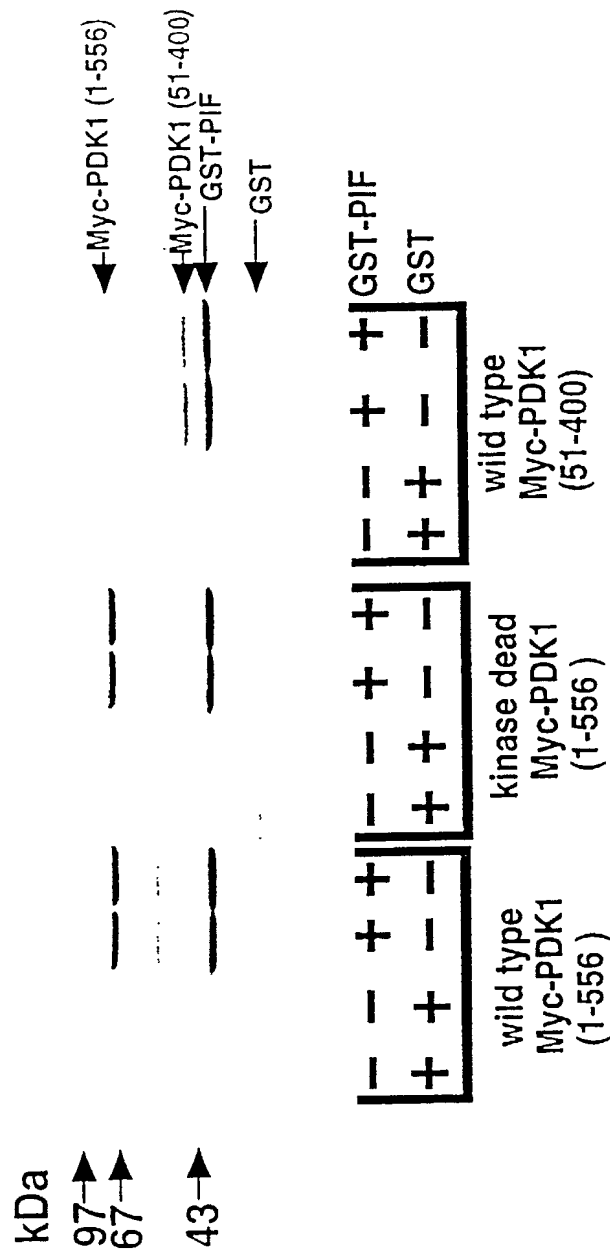


Fig 2 (page 1 of 3)

Myc antibody immunoprecipitate

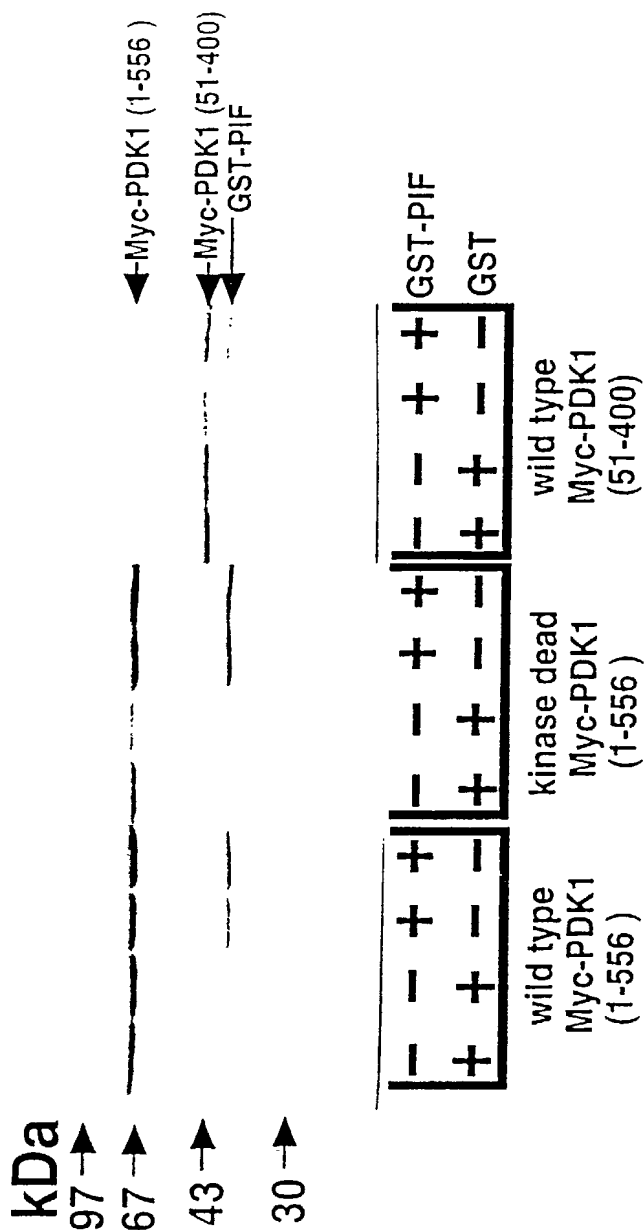
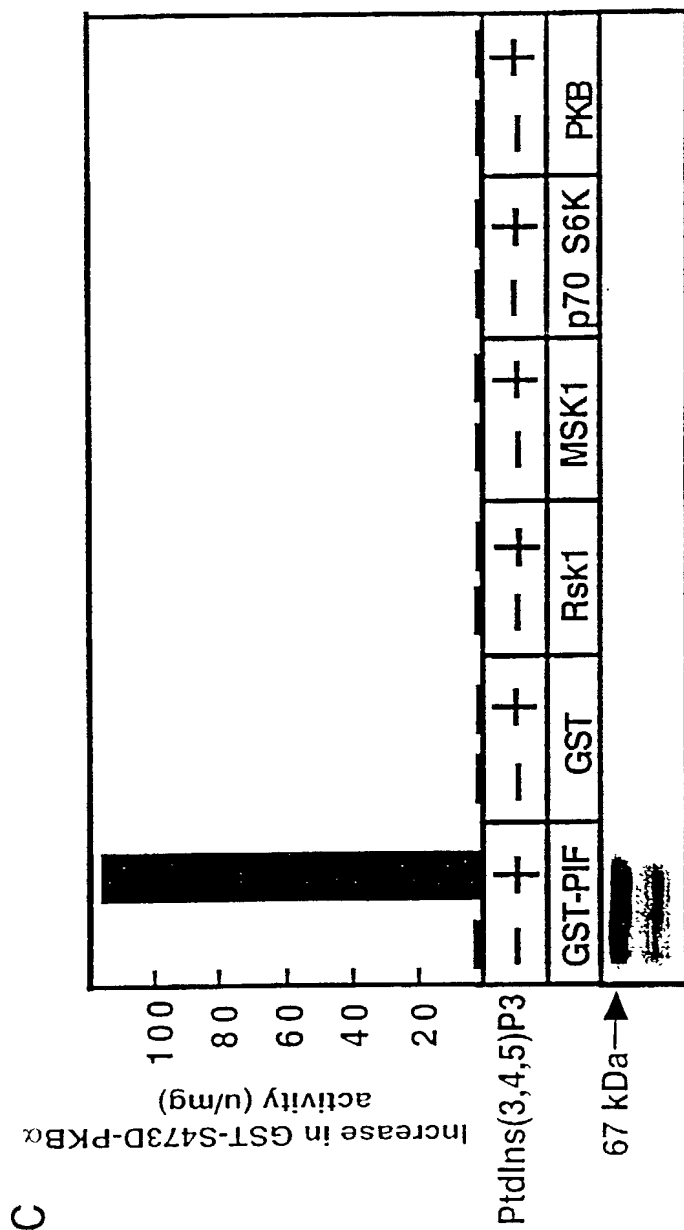


Fig 2 (page 2 of 3)



Western blot for endogenous PDK1

Fig 2 (page 3 of 3)

69/937009

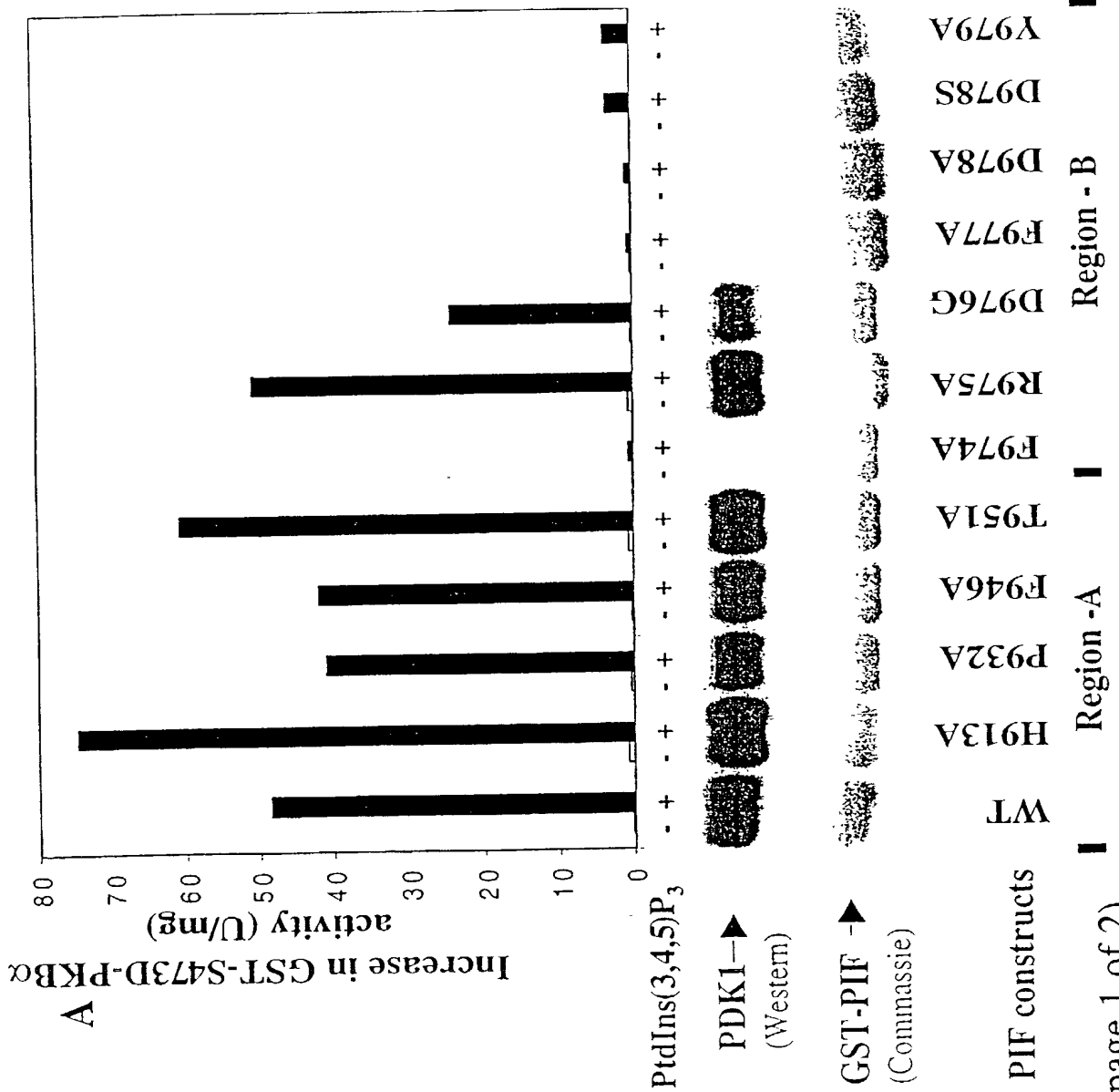


Fig 3 (page 1 of 2)

5/22

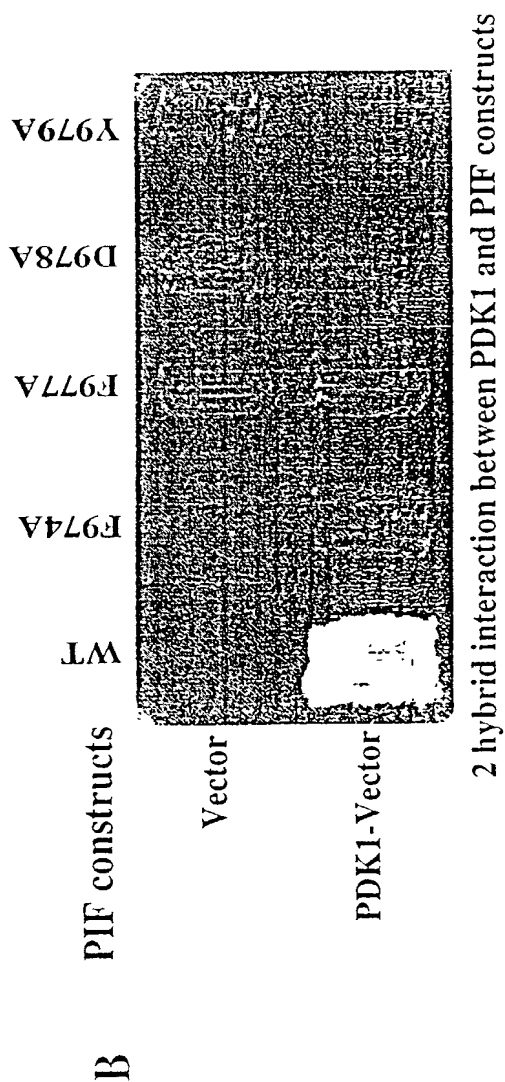


Fig 3 (page 2 of 2)

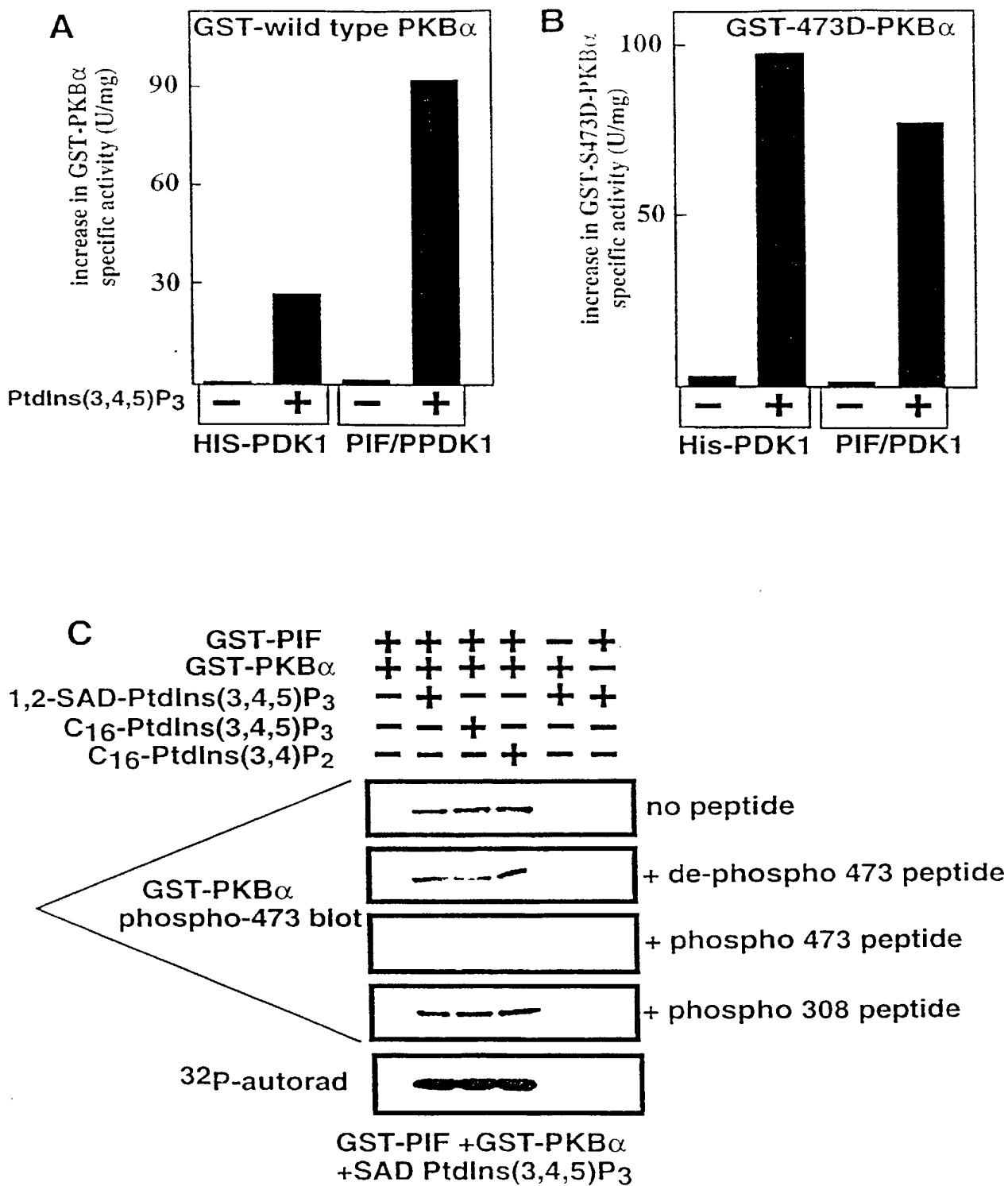


Fig 4 (page 1 of 2)

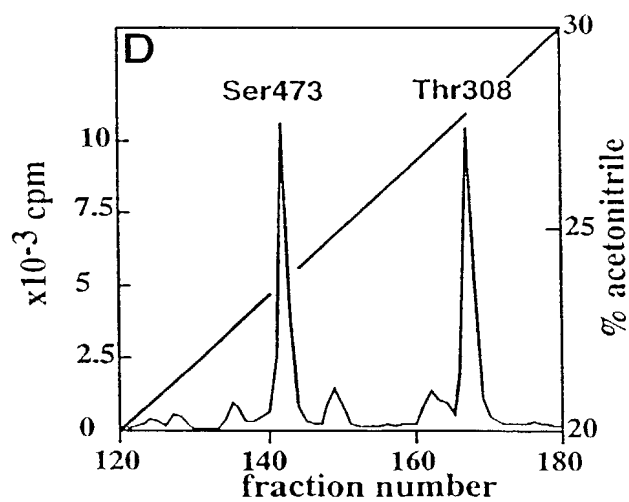


Fig 4 (page 2 of 2)

8/22



**A**

	Control	Non specific antibody	PDK1 protein antibody	PDK1 peptide antibody	PDK1 peptide antibody + Peptide
1,2-SAD-PtdIns(3,4,5)P <sub>3</sub>	- + - -	- + - -	- + - -	- + - -	- + - -
C16-PtdIns(3,4,5)P <sub>3</sub>	- - + -	- - + -	- - + -	- - + -	- - + -
C16-PtdIns(3,4)P <sub>2</sub>	- - - +	- - - +	- - - +	- - - +	- - - +
	- - - -	- - - -	- - - -	- - - -	- - - -

PKB $\alpha$  phospho-Ser473 blot in supernatant of immunoprecipitates

**B**

H913A	P932A	F946A	T951A	Q971A	F974A	R975A	D976G	F977A	D978A	D978S	Y979A
- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +	- +
1,2-SAD-PtdIns(3,4,5)P <sub>3</sub>											
PKB $\alpha$ phospho 473 blot											

Region A

Region B

935-FRDFDY-940

PDK2 motif in PRK2

Fig 5

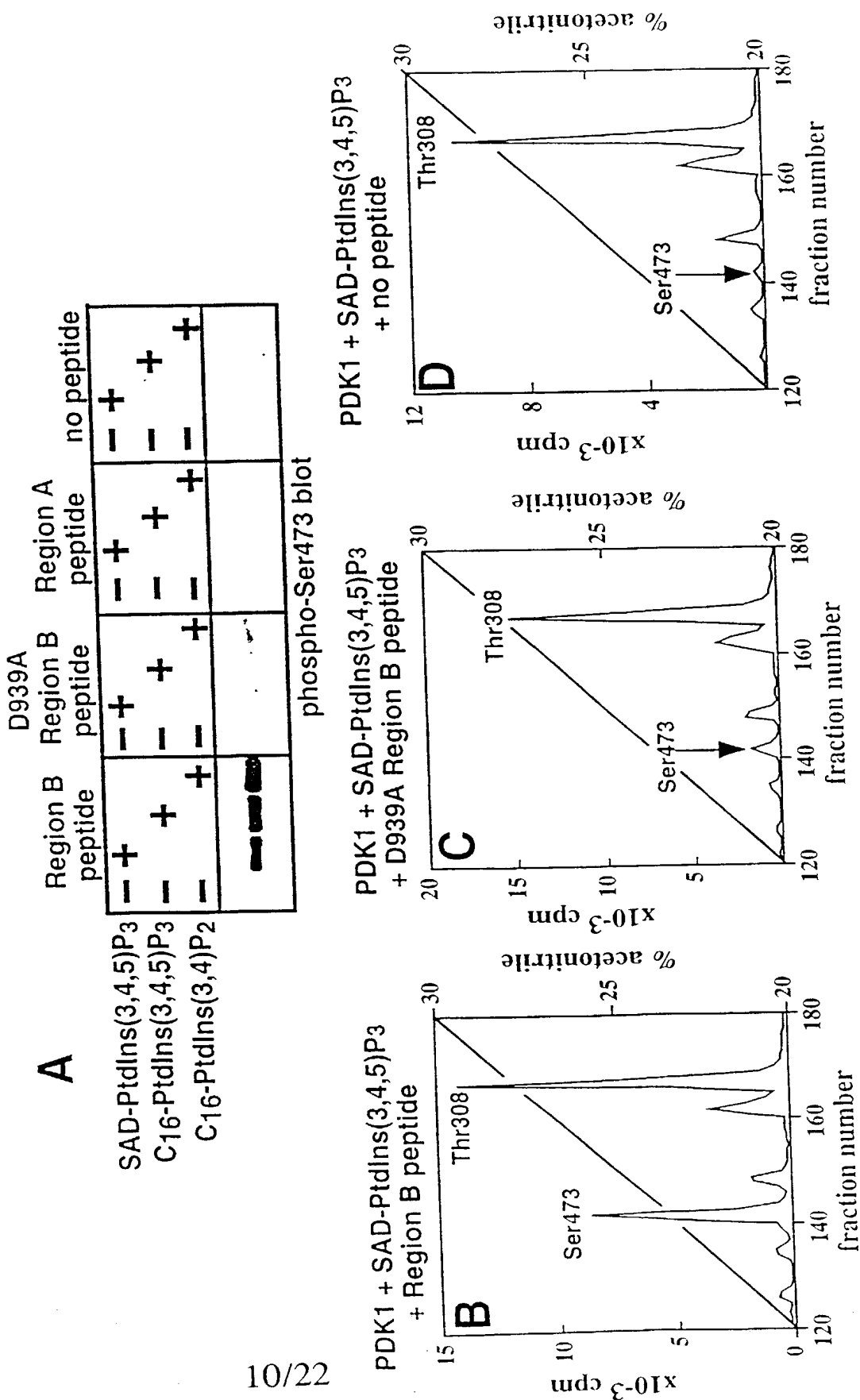
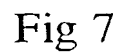


Fig 6



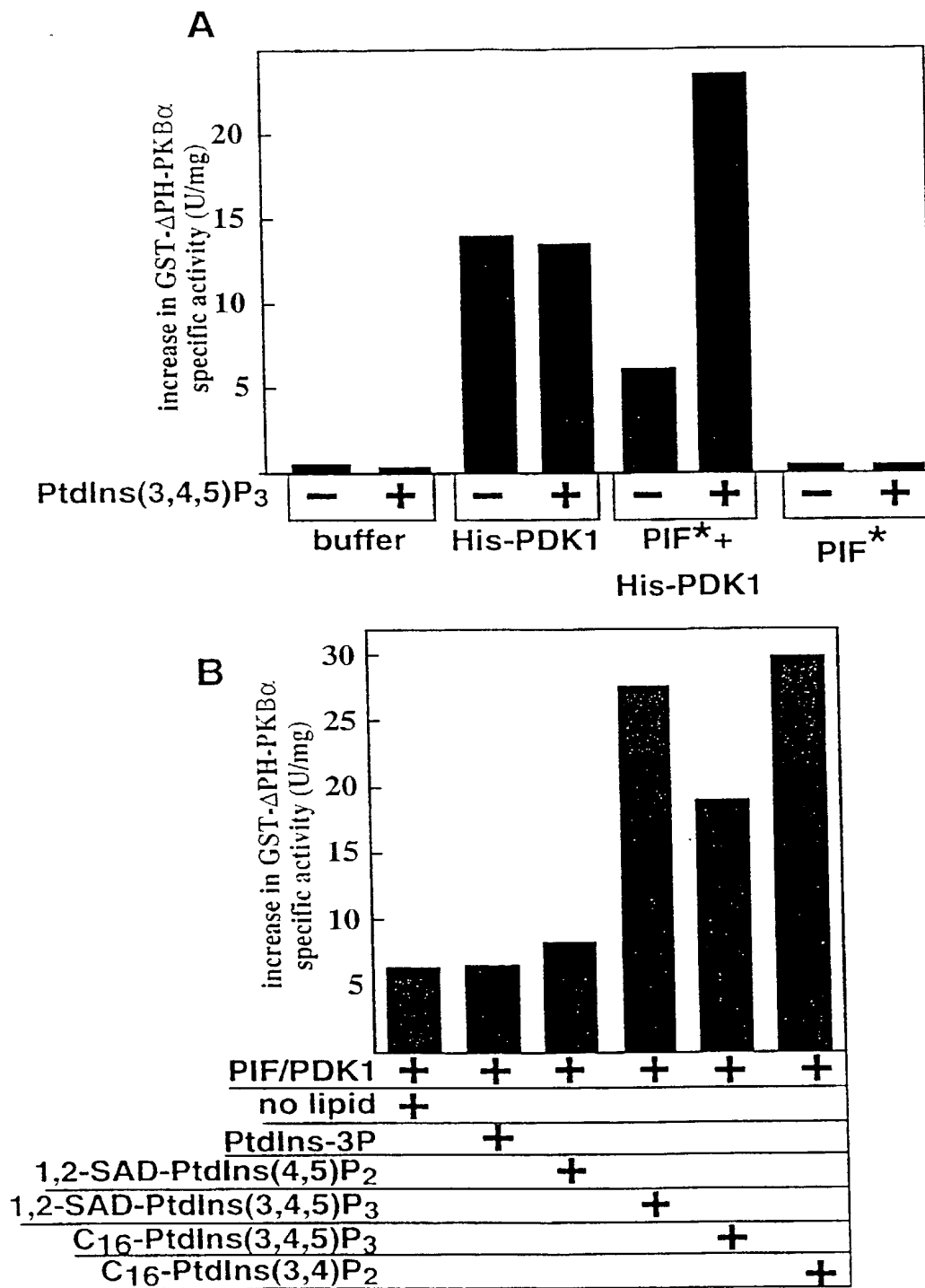


Fig 8

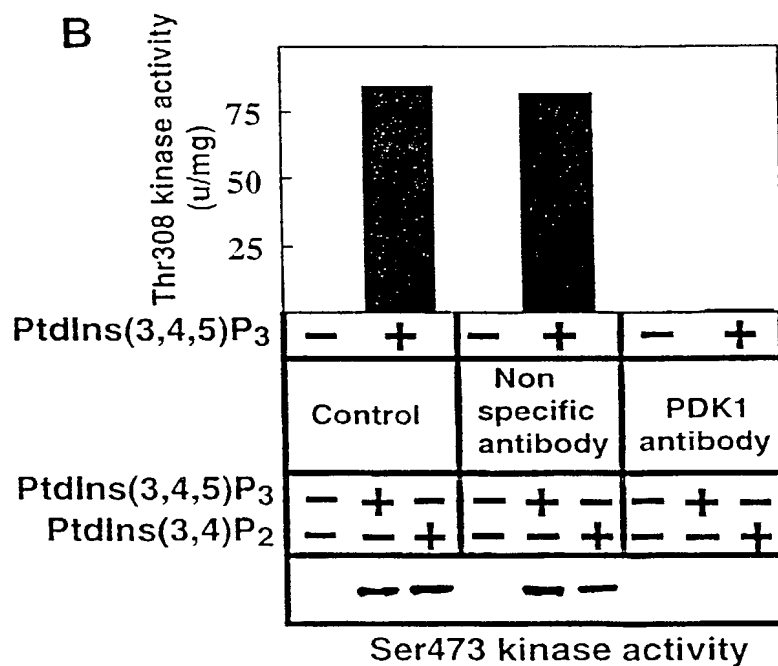
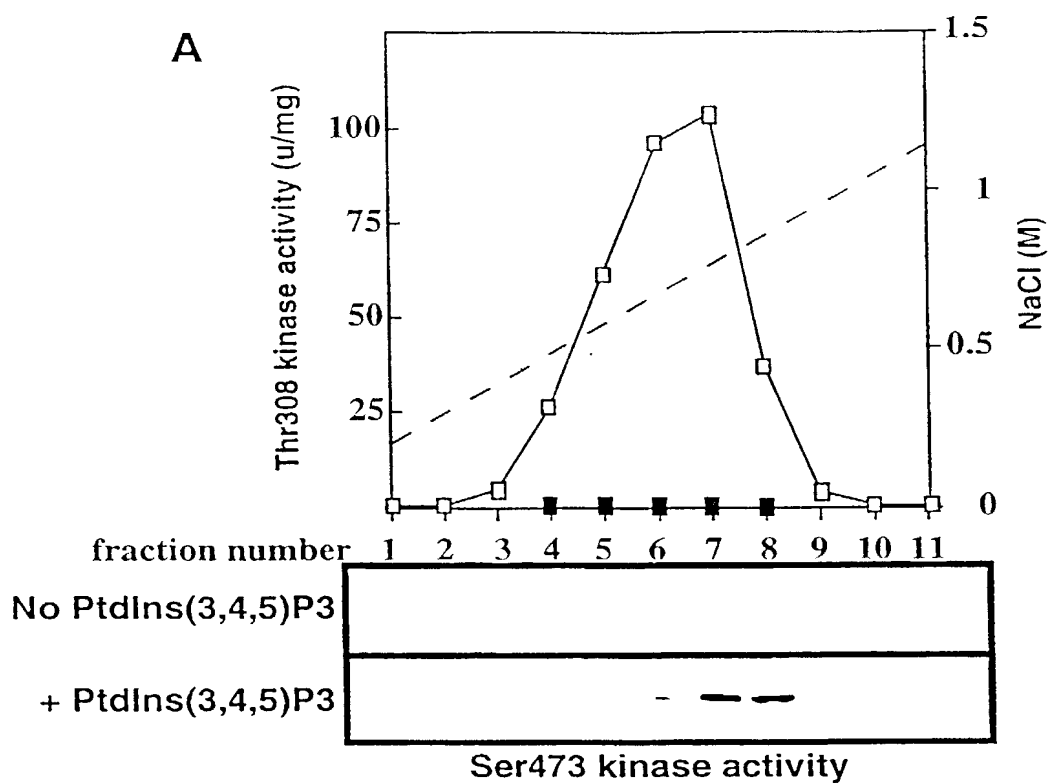


Fig 9

Fig 10

MARTTSQLYDAVPIQSSVVLCSPPSPSMVRTQTESSTPPGIPGGSRQGPAM  
DGTAAEPRPGAGSLQHAQPPPQPRKKRPEDFKFGKILGEGSFSTVVLAREL  
ATSREYAIKILEKRHIKENKVPYVTRERDVMSRLDHPFFVKLYFTFQDDE  
KLYFGLSYAKNGELLKYIRKIGSFDETCTRFYTAEIVSALEYLHGKGIHR  
DLKPENILLNEDMHIQITDFGTAKVLSPESKQARANSFVGTAQYVSPPELLT  
EKSACKSSDLWALGCIIYQLVAGLPPFRAGNEYLIFQKIIKLEYDFPEKFF  
PKARDLVEKLLVLDATKRLGCEEMEGYGPLKAHPFFESVTWENLHQQTTPPK  
LTAYLPAMSEDDDCYGNYDNLLSQFGCMQVSSSSSSSHLSASDTGLPQRS  
GSNIEQYIHDLDNSNFELDLQFSEDEKRLLEKQAGGNPWHQFVENNLILK  
MGPVDKRKGLFARRRQLLLTEGPHLYYVDPVNKVLKGEIPWSQELRPEAKN  
FKTFFVHTPNRTYYLMDPSGNAHKWCRKIQEVWRQRYQSHPDAAVQ

PDK1 sequence

## Fig 11 (page 1 of 2)

## Human PRK2 sequence information:

LOCUS 1000125 984 aa 04-FEB-1999  
 DEFINITION PRK2  
 ACCESSION 1000125  
 PID g1000125  
 DBSOURCE GENBANK: locus HSU33052, accession U33052  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (residues 1 to 984)  
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.  
 TITLE Identification of multiple, novel, protein kinase C-related  
 gene products  
 JOURNAL FEBS Lett. 356 (1), 5-8 (1994)  
 MEDLINE 95080426  
 REFERENCE 2 (residues 1 to 984)  
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.  
 TITLE Cloning and expression patterns of two members of a novel  
 protein-kinase-C-related kinase family  
 JOURNAL Eur. J. Biochem. 227 (1-2), 344-351 (1995)  
 MEDLINE 95154310  
 REFERENCE 3 (residues 1 to 984)  
 AUTHORS Palmer, R.H.  
 TITLE Direct Submission  
 JOURNAL Submitted (02-AUG-1995) Ruth H. Palmer, Protein  
 Phosphorylation,  
 ICRF, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK  
 COMMENT Method: conceptual translation.  
 FEATURES  
 Location/Qualifiers  
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 /db\_xref="taxon:9606"  
 /clone\_lib="cDNA library from human DX3 cell line  
 (B-cell  
 lineage)"  
 Protein 1..984  
 /product="PRK2"  
 CDS 1..984  
 /note="lipid-activated, protein kinase C-related,  
 serine/threonine protein kinase"  
 /coded\_by="U33052.10..2964"

## ORIGIN

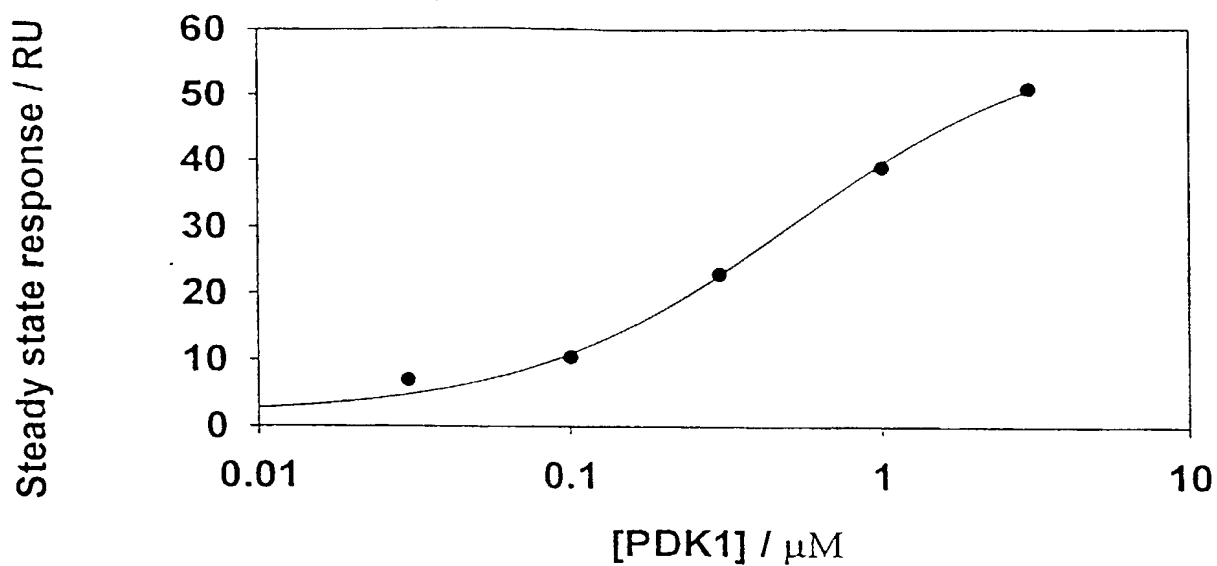
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 61 kikegaenlr kvttddksla yvdnilkksn kkleelhhkl qelnahivvs dpeditdcpr  
 121 tpdtpnnndpr cstsnrika lqkqldielk vkqgaenmiq mysngsskdr klhgtaqql  
 181 qdsktkievi rmqilqavqt nelfadnakp visplelrme elrhhfrief avaegaknvm  
 241 klqsgskvtd rkalseaqar tnessqkldi lkysleqrln evpknnoksr illeelslva  
 301 asptlsprqs mistqnqyst lskpaaltgt levrlmgcqd ilenvpgrsk atsvallpgws  
 361 psetrssfms rtsksksgss rnllktddls ndvcavlkld ntvvgqtswk pslnqswdqk  
 421 ftleldrsre leisvywrw rslcavkflr ledflnqrh gmclylepqg tifaevtffn

481 pvierrpklq rqkkifskqq gktflrāpqm niniatwgrl vrraiptvnh sgtfspqapv  
 541 pttvpvvdvr ipqlappasd stvtkldfdl epepppappr asslgeides selrvldipg  
 601 qdsetvfdiq ndrnsilpks qseykpdtpq sgleysgiqe ledrrsqqrq qfnlqdfrc  
 661 avlgrgntgk villaeykntn emfaikaikk gdivardevd slmcekrife tvnsvrhpfl  
 721 vnlfacfqtk ehvcfvmeya aggdmmhnh tdvfseprav fyaacvvlgl qylhehkiv  
 781 rdlkldnlll dtegfvkiaf fglckegmgy gdrststfcgt peflapevlt etsytravdw  
 841 wglgvliiem lvgespfpgd deeevfdsv ndevryprfl steaisimrr llrnperri  
 901 gasekdaedv kkhpfrrld wsalmdkkvk ppfiptirgr edvsnfdded tseapiltpp  
 961 repriiseee qemirddfy adwc

Fig 11 (page 2 of 2)



## (A) HisPDK1 binding to GST-PIF



## (B) HisPDK1 binding to PIF region B peptide

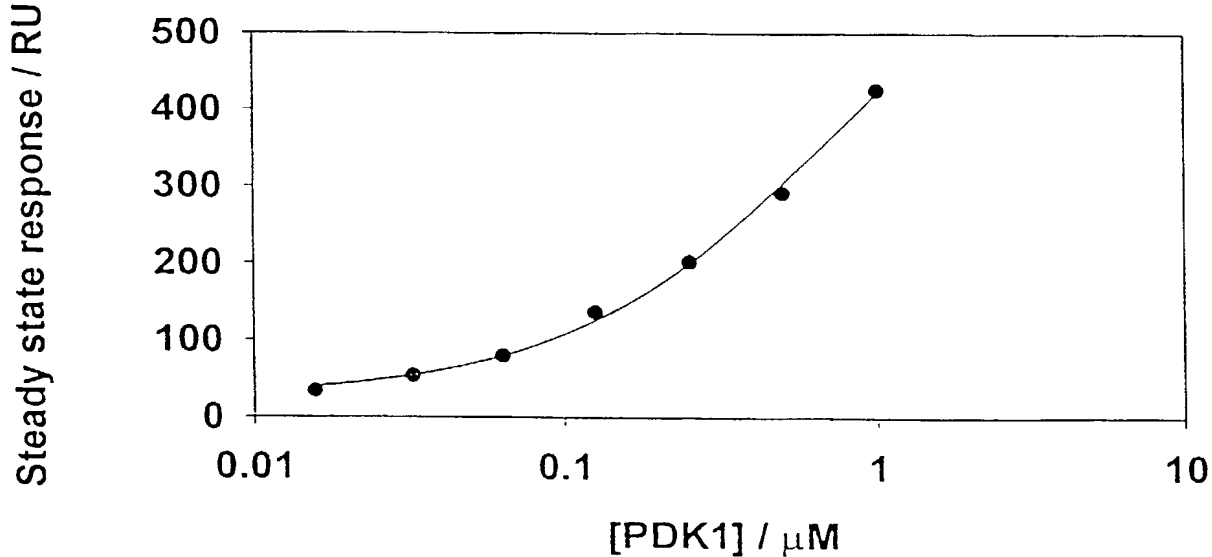


Fig 12 (page 1 of 2)

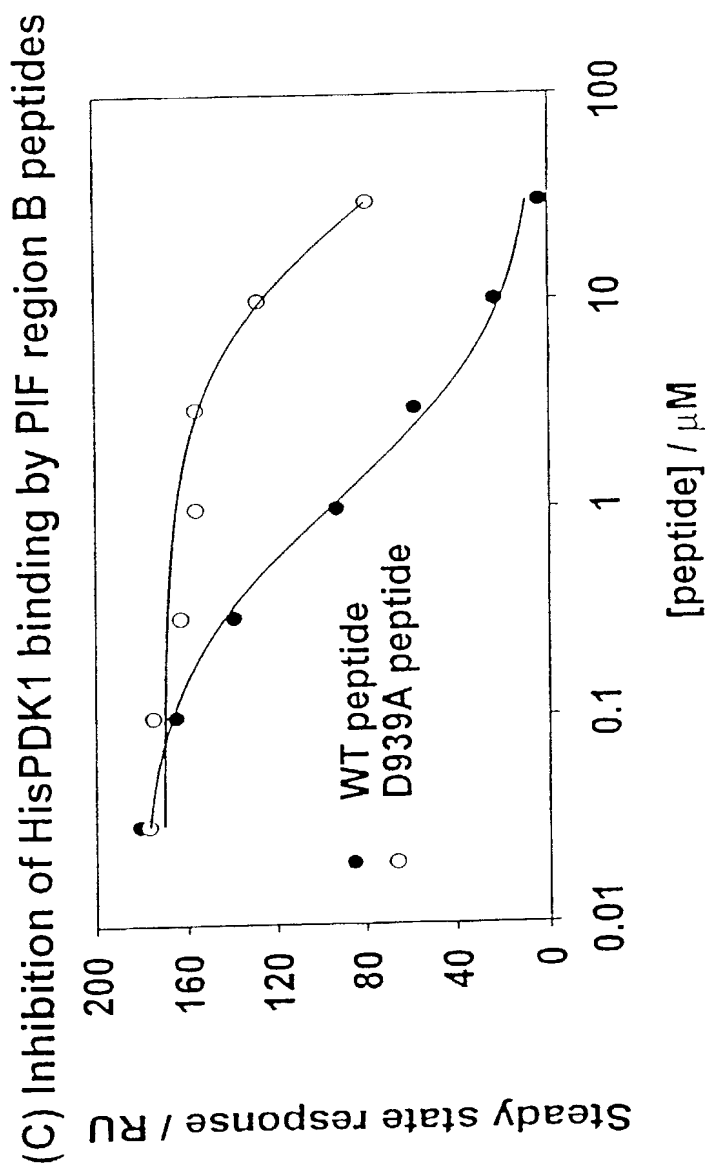


Fig 12 (page 2 of 2)

**A**

	Wild type GST-PDK1			kinase dead GST-PDK1		
GST-PIF*	+	+	+	+	+	+
SAD-PtdIns(3,4,5)P <sub>3</sub>	+	+	+	+	+	+
C16-PtdIns(3,4,5)P <sub>3</sub>	-	+	+	-	+	+
C16-PtdIns(3,4)P <sub>2</sub>	-	-	+	-	+	+

phospho-Ser473 blot

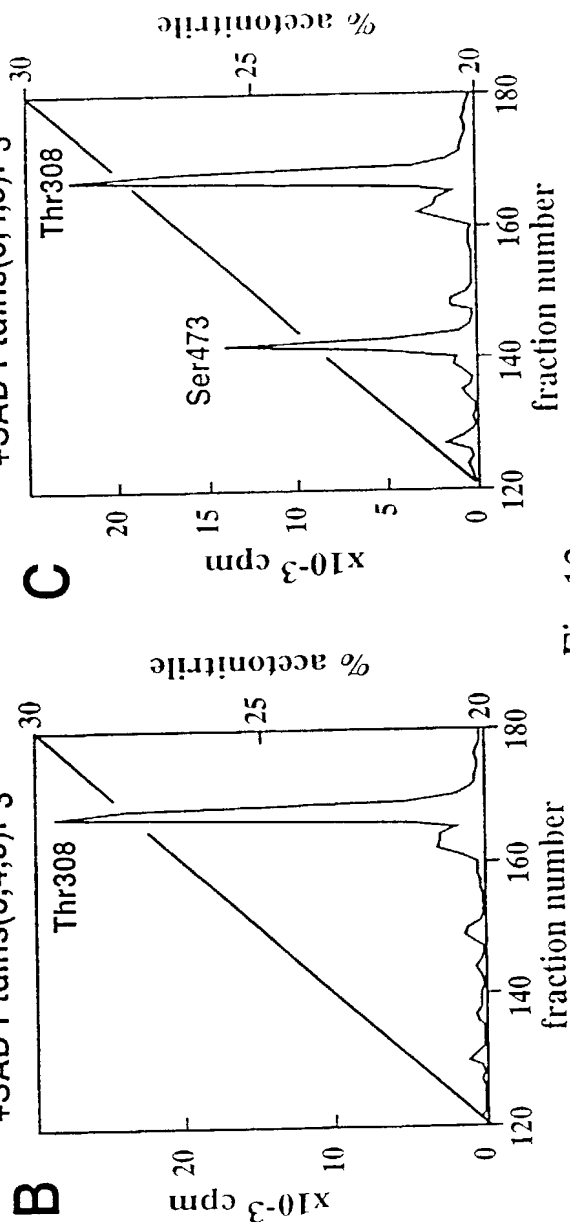


Fig 13

## Fig 14

## Human PRK1 sequence information:

LOCUS 2136028 942 aa 02-JUL-1996  
 DEFINITION protein kinase PRK1 - human.  
 ACCESSION 2136028  
 PID g2136028  
 DBSOURCE PIR: locus I53327  
 summary. #length 942 #molecular-weight 103989 #checksum 8328.  
 PIR dates: 02-Jul-1996 #sequence\_revision 02-Jul-1996  
 #text\_change  
 02-Jul-1996.  
 KEYWORDS .  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Mammalia, Eutheria; Primates; Catarrhini;  
 Hominidae; Homo.  
 REFERENCE 1 (residues 1 to 942)  
 AUTHORS Palmer, R.H., Ridden, J. and Parker, P.J.  
 TITLE Cloning and expression patterns of two members of a novel  
 protein-kinase-C-related kinase family  
 JOURNAL Eur. J Biochem. 227 (1-2), 344-351 (1995)  
 MEDLINE 95154310  
 FEATURES Location/Qualifiers  
 source 1..942  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 Protein 1..942  
 /product="protein kinase PRK1"

## ORIGIN

1 masdavgsep rswsilleqlg lagadlaapg vqqglelere rlrreirke lkegaenlr  
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 121 satnlsvrag lekqlalelk vkqgaenmiq tysngstkdr kliltaqqml qdsktkidli  
 181 rmqlrralqa dqlenqaapd dtqgspdlga velrieelrh hfrvehavae gahnvlrlis  
 241 aakapdrkav seaekltres nqklgllrea lerrlgelpa dhpkgllre elaaassaaf  
 301 strlagpfpa thystlckpa pltgtlevrv vgcrlpeti pwnptpsmgg pgtpdsrppf  
 361 lsrparglys rsgslsgrss lkaeaentse vstvlkidnt vvgqtswkpc gpnawdqsft  
 421 lelerarele lavfwrdrqg lcalkfkle dldnerhev qldmepggcl vaevtfirnpv  
 481 ieriprlrrq kkifskqgk afqrarqmni dvtwvrlir rlipnatgtg tfspgaspgs  
 541 earttgdisv eknlgtddsd sspqkssrdp psspslssp igestapelp setqetpgpa  
 601 lcsplrkspl tledfkflav lgrghfgkvl lsefrpsgel faikalkkgd ivardevesl  
 661 mcekrilaav tsaghpflvn lfgcfqtpch vcfvmeysag gdlmlhihsd vfsepraify  
 721 sacvvlgqlf lhekivyr d lkldnllldt egyptkiadfg lckegmggyd rtstfcgtpe  
 781 flapevlttdt sytravdwg lgvilyemlv gespfpgdde eevfdisvnd evryprflsa  
 841 eaigimrrll rnpperrls serdaedvkk qpffrtlgwe allarrlppp fvptlsgrtd  
 901 vsnfdeeftg eaptlspprd arpltaaeqa afldfdvav gc

## Fig 15 (page 1 of 2)

## Human PKC zeta sequence information:

LOCUS 478322 592 aa 28-FEB-1997  
 DEFINITION protein kinase C (EC 2.7.1.-) zeta - human.  
 ACCESSION 478322  
 PID g478322  
 DBSOURCE PIR: locus JN0877  
 summary: #length 592 #molecular-weight 67731 #checksum 87.  
 genetic: #gene GDB:PRKCZ ##cross-references GDB:128040.  
 superfamily: protein kinase C zeta; protein kinase C zinc-binding  
 repeat homology; protein kinase homology.  
 PIR dates: 03-May-1994 #sequence\_revision 03-May-1994  
 #text\_change 28-Feb-1997.  
 KEYWORDS ATP; phorbol ester receptor; phosphotransferase;  
 serine/threonine-specific protein kinase; zinc.  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Mammalia; Eutheria; Primates; Catarrhini,  
 Hominidae; Homo.  
 REFERENCE 1 (residues 1 to 592)  
 AUTHORS Kochs,G., Hummel,R., Meyer,D., Hug,H., Marme,D. and  
 Sarre,T F.  
 TITLE Activation and substrate specificity of the human protein  
 kinase C alpha and zeta isoenzymes  
 JOURNAL Eur. J. Biochem. 216 (2), 597-606 (1993)  
 MEDLINE 93387312  
 REFERENCE 2 (residues 1 to 592)  
 AUTHORS Barbee,J.L., Deutscher,S.L., Loomis,C.R. and Burns,D.J.  
 TITLE The cDNA sequence encoding human protein kinase C-zeta  
 JOURNAL Gene 132 (2), 305-306 (1993)  
 MEDLINE 94040779  
 REFERENCE 3 (residues 1 to 592)  
 AUTHORS Hug, H.  
 TITLE Direct Submission  
 JOURNAL Submitted (??-SEP-1992) to the EMBL Data Library  
 FEATURES Location/Qualifiers  
 source 1..592  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 Protein 1..592  
 /product="protein kinase C zeta"  
 /EC\_number="2.7.1.-"  
 Region 131..180  
 /note="protein kinase C zinc-binding repeat homology  
 #label KZ1"  
 /region\_name="domain"  
 Region 250..518  
 /note="protein kinase homology #label KIN"  
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 Region 258..266  
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 /region\_name="region"  
 Site 281  
 /note="Lys"

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ORIGIN

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121 rwrklyrang hlfqakrfnr raycgqcser iwglarqgyr cinckllvhk rchglvpltc
181 rkhdmsvmps qeppvddknc dadlpseetd giayissrk hdsikddsed lkpvidgmdg
241 ikisqglglq dfdlirvigr gtyakvlvr lkkndqiyam kvvkkelvhd dedidwvqte
301 khvfcqassn pflvglhscf qttsrlflvi eyvnggd1mf hmqrqrklpe eharfyaaei
361 cialnflher giiyrdlkld nvlldadgh1 kldygmcke glgpgdtst fcgtpnyiap
421 eilrgeeygf svdwwalgv1 mfemmagrsp fdiitdnpdm ntedylfqvi lekpiriprf
481 lsvkashvlk gflnkdpker lgrcpqtgfs dikshaffrs idwdllekkq alppfqpqit
541 ddygldnfdt qftsepvqlt pddedaikri dqsefegfey inplllstee sv

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Fig 15 (page 2 of 2)